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(54) Title: METHODS OF PRODUCING AND SCREENING COMPLEX CHEMICAL LIBRARIES

(57) Abstract

Methods for producing complex mixtures of compounds are provided, where in the first or subsequent stage, a plurality of vessels are employed. In each vessel is present a polyfunctional core molecule, where the functionalities have similar reactivities, and adduct molecules which react at a similar rate under the conditions of the reaction. Each vessel has a set of overlapping but different adduct molecules, so as to provide a diverse set of products. The products are then screened for activity, where a particular reaction mixture is then analyzed by repeating the process of dividing the adducts into overlapping but different sets in different vessels and then screening the vessels for activity. In this manner, a single compound or group of compounds which can be analyzed and screened can be obtained, so as to define the compound(s) which demonstrates the designated activity.

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METHODS OF PRODUCING AND SCREENING COMPLEX CHEMICAL LIBRARIES

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of application Serial No. 08/151,727, filed November 12, 1993.

INTRODUCTION

Technical Field

The field of this invention concerns the preparation of complex mixtures of organic compounds, identification of activity, and identification of individual compounds having the activity.

Background

Synthetic organic compounds play an extraordinary role in the well being of modern society. Synthetic organic compounds find application as therapeutics, as pesticides, as additives in a wide variety of context, as dyes, as well as in many other environments. Over the years numerous synthetic organic compounds have been produced, particularly those compounds, where there has been some *ab initio* reason for believing that the compound would have an activity of interest. This belief could be predicated upon an activity of a natural occurring compound, some insight into the nature of the target, or the like. The breadth of compounds that have been produced is quite staggering in number, but the variety of opportunities in producing organic compounds, due to the versatility of carbon and the numerous groups that can be

used to functionalize carbon atoms, leaves a vast unexplored area.

While increasing sophistication in instrumentation and knowledge has expanded the ability to produce novel synthetic organic compounds, it has also greatly increased the cost. Thus, producing individual organic compounds to develop a class of compounds for screening has become an extremely expensive exercise, particularly where there is no assurance that any of the candidate compounds of the class will be found to be active. There has, therefore, been substantial activity in developing alternative protocols for producing candidate compounds for investigation as to their physiological activity, physical characteristics, and utility.

One approach has been commonly called "combinatorial chemistry." This approach employs numerous reagents in a series of steps, which allows for the simultaneous production of large numbers of compounds.

Combinatorial chemistry is a very powerful strategy. Combinatorial libraries make large additions to the chemical databases, typically used in the pharmaceutical industry; combinatorial chemistry effectively combines all the laborious tasks in traditional drug discovery, e.g. collection and testing of natural product samples, isolation and purification of active ingredients, structure determination and synthesis, into one rapid series of steps; since combinatorial chemistry creates such a large pool of potential active compounds, the candidate compounds are often more specific than those found in nature or synthesized by traditional means; small organic compounds make up the vast majority of drugs and other physiologically active compounds, and large numbers of small organic molecules can be made over short periods of time.

The methodology requires an accessible means for determining the nature of a product which is active as to a particular property or characteristic. The requirement

for identification of a product which is found to be active has severely limited the approaches used to produce large libraries.

One approach has been the preparation of oligomers, such as oligopeptides and oligonucleotides. These approaches have involved a wide variety of methods for producing the oligomers and determining their structure, as well as screening the oligomers for activity. However, despite the very substantial efforts involved and the numerous protocols for their preparation and screening, there have been no reported significant successes in therapeutically useful drug development. One of the problems is that the oligomers are relatively flexible and it is generally believed that fairly rigid molecules will be required for binding, where a target molecule is involved, to achieve the end purpose. The linear biopolymers, such as oligonucleotides, peptides and peptidomimetics suffer from poor oral bioavailability, being too large to be absorbed and too readily degraded physiologically. Efforts have therefore been directed to synthetic techniques for synthesizing small, organic molecules. Bunin and Ellman, *J. Am. Chem. Soc.* 114, 10997 (1992), reported a combinatorial approach to the synthesis of diazepams. By using a few steps and a relatively limited repertoire, Ellman was able to synthesize a library of diazepam compounds. This approach is fairly restricted in the number and type of compounds which can be produced, since the products must be produced one at a time.

Recently, an approach to combinatorial libraries was reported by Stills' group involving particles, where reactions are carried out in separate vessels on particles using different reagents in the different vessels, and the particles in the vessels tagged with chemical compounds. Ohlmeyer et al., *Proc. Natl. Acad. Sci. USA* 90, 1464-1468 (1993). After each series of reactions, all of the particles are combined together and then separated into

separate vessels for the next series of reactions and tagging. After screening the compounds which are produced, those particles which carry an active compound, can then be analyzed as to the synthetic sequence which has been employed with that particle by reading the tags.

Because of the great opportunities that combinatorial chemistry affords, it is important that new approaches be devised for the preparation of libraries, which can afford advantages over the presently existing protocols.

Relevant Literature

Review articles include Baum, Chemical and Engineering News, 72, 20-26, (1994), Baum, *ibid* 71, 33-34 (1992), and Amato, *Science* 257, 330-331 (1992). Patents of interest include U.S. patent nos. 5,182,366; 5,270,170; and 5,252,296. PCT applications of interest include WO94/06017; WO94/04558; WO94/02515; WO93/20242; WO93/19205; W093/06121; WO92/00091; and WO91/17283.

Other references of interest include Fodor et al., *Science* 251, 767 (1991); Lam et al., *Nature* 354, 82 (1991); Houghten et al., *Nature* 354, 84 (1991); Blake and Davis, *Bioconjugate Chem.* 3, 510 (1992); Schultz et al., *Science* 261, 1303 (1993); Brenner and Lerner, *Proc. Natl. Acad. Sci. USA* 89, 5381 (1992); and Janda et al., *J. Am. Chem. Soc.* 115, 9812 (1993).

SUMMARY OF THE INVENTION

A method for producing a library of related molecules is provided employing a polyfunctional core molecule and a plurality of adduct molecules capable of reacting with the core molecule functionalities. A plurality of non-overlapping adduct subset units are defined to be used to define the adduct reaction set. As a first or intermediate step, in each of a series of vessels one of overlapping different sets of adduct molecules comprised of less than the total number of subset units are added to the core molecules under reaction conditions, where

usually the reaction rate in each vessel of each of the adduct molecules is substantially normalized. The content of each vessel is then screened for activity as to one or more utilities. For each vessel which shows activity, a second series of reactions may be carried out using the core molecules and a set of adduct molecules having a reduced number per vessel of adduct molecules coming from within the set which was active. This set may be selected to ensure that all the molecules produced in the active set are produced or, by comparing the results of the overlapping sets, one or more of the adduct molecules may be excluded from the set as not being involved in an active product. A screening program is provided to rapidly narrow a large number of candidate molecules to a relatively small number of candidate molecules, which may then be characterized by physical or other means as to the identity of the active compound(s).

DESCRIPTION OF SPECIFIC EMBODIMENTS

In accordance with the subject invention, complex mixtures, which are non-polymeric synthetic organic molecules are produced, where a plurality of vessels are employed, each vessel containing the same polyfunctional core molecule. The polyfunctional core molecule may have the same functionalities [poly(mono)functional] or different functionalities having similar reactivity to the adducts [poly(multi)functional]. To each vessel is added a set of reactants ("adducts") capable of reacting with the functionalities present on the core molecule. Non-overlapping subsets of adducts are chosen to define overlapping different sets of adducts. The sets are selected to provide all possible combinations of products resulting from reactions with the members of all of the sets. The reaction is allowed to proceed in each of the vessels to at least substantial completion. The conditions of the reaction, including the concentrations of each of the adducts, are selected so as to

substantially normalize the rate of reaction of each of the adducts with the core molecule functionality and to minimize undesired side reactions. After completion of the reaction, all or aliquots of a reaction mixture from a single vessel may then be screened for activity as to a particular purpose. Any activity may be considered or a minimal threshold activity may be selected for a particular mixture as defining activity.

As to those vessels where the activity exceeds the selected threshold activity, a second series of reactions may be carried out. Once again, a plurality of vessels is employed, where the core molecule is present in each vessel, and individual sets of adducts are added to each vessel, where the sets have fewer adducts than the previous set, and may provide less than all of the compounds which were produced in the previous reaction vessel up to all of the compounds. Where less than all of the compounds will be produced, this may be as a result of analysis of activity of the various mixtures, demonstrating that certain combinations will not be active or allowing for the absence of certain combinations, where lack of activity in a vessel would indicate that the compound(s) which was not produced provided the activity in the previous vessel. This process may be repeated as many times as necessary to obtain a single product or a mixture of products which can be analyzed, so as to identify the individual products in the mixture, where the individual synthesis of all the products is not onerous.

The number of adduct molecules will usually be at least 6, more usually at least 8, generally at least about 12, usually not exceeding 200, more usually not exceeding 100.

The step or stage comprising a plurality of adducts, where sets of adducts are used in different reaction vessels with the same core molecules, may be the first step or an intermediate step. While one may carry out a single reaction in a single vessel with a set of all the

adducts, and then screen the entire mixture for activity, generally this will not be done. More usually, one will begin as a first step using a plurality of vessels, at least two, more usually at least three, preferably at least about five, and usually not more than 300, more usually not more than 200, and preferably not more than 100. The number of vessels will be dictated by the number of functionalities on the core molecule, the number of adducts, the desired complexity of the mixtures, the sensitivity of the assay(s) for screening activity, the amounts of products needed, and the like.

Usually, the system will provide for the preparation of at least about 100 compounds, more usually at least about 200 compounds, preferably at least about 500 compounds, frequently at least about 1,000 compounds, usually fewer than about 5×10^6 compounds, more usually fewer than about 1×10^6 compounds. While for the most part it may be assumed that the primary products will be the per-substituted core molecule, one may anticipate that in many situations, there will be small but identifiable amounts of core molecules which have reacted at fewer than the total number of functionalities present.

In carrying out the subject invention, a "primary" list of adduct molecules is made. This list defines the variety of products which may be achieved in relation to the core molecule. As a convenient tool, this list may be divided into non-overlapping subset units, where the number of units is equal to or greater than the number of reactive functionalities on the core molecule. These units are then combined to provide different overlapping sets of adducts for reaction in different vessels, so that the total number of possible products from the adducts is obtained. Each vessel will have a different mixture of products, having a proportion of products which overlap the products in other vessels. While still obtaining the total number of products available from the number of adducts used, screening is improved in that fewer than the

total number of products is present in each vessel, allowing for fewer iterative steps to define active compounds, with less chance for interactions between compounds confusing the results of the assays. As one finds activity in a particular vessel, one can then repeat the process, using the list of adduct molecules used in that particular vessel to define the total number of adduct molecules which are to be distributed among the plurality of vessels in accordance with the previously described strategy in the next step.

The total number of products produced by the subject method is indicated by the following:

$$R^i\text{-}X + Z\text{-}(Y)_v \dashrightarrow Z(\text{-}W\text{-}R^i)_v$$

where i=1 to n.

The equation indicates that there are "n" different adduct molecules, (R^i) where i=1 to n, which will be reacted with a core molecule having "v" reactive functionalities ("Y"). The products all have the same core moiety "Z," but have random covalently bonded adduct moieties, where the R groups attached to the core moiety may be the same or different. X is a reactive functionality, which reacts with Y to form a covalent bond. W is the linking group formed from the reaction of X and Y.

Illustrative of the reaction situation would be a core molecule with four reactive functionalities. When the core molecule has no axis or plane of symmetry, each of the reactive centers is unique and the number of possible products is given by "n^v". If the reaction utilizes 50 adduct reagents, then the number of expected products is $50^4 = 6{,}250{,}000$.

The screening strategy should efficiently narrow the number of adduct molecules used at each step, while ensuring that each of the possible products produced by the original adduct set is formed in at least one of the subsets. To accomplish this, the primary adduct set, S_1, is divided into "k" non-overlapping partial subsets, p_i,

with equal numbers of adducts (≥ 1 adduct per subset), where a subscript "i" denotes an integer of from 1 to k (k being greater than the number of reactive functionalities on the core molecule). Secondary subsets s_{2j}, are then prepared by making each possible combination or union set of "v" partial subsets (v is the number of reactive functionalities on the core molecule and the subscript j is an integer from 1 to m, where m is the number of subsets). The secondary subsets are then used to synthesize secondary product mixtures for screening.

For example, if a trifunctional asymmetrical core molecule is reacted with a primary adduct set of 12 different adduct molecules, the following methodology can then be used to identify an active compound from among the $12^3 = 1728$ products. The original adduct set of 12 adduct molecules (termed A,B,C,D,E,F,G,H,I,J,K,L) is divided into a number of non-overlapping partial subset units. Here the number of partial subset units, k, is selected to be greater than the number of reactive functionalities on the core molecule. In this example using a core molecule with three reactive functionalities, the adduct list is divided into k=4 partial subsets p_i.

$$s_1 = \{A, B, C, D, E, F, G, H, I, J, K, L\}$$

$$p_1 = \{A, B, C\}$$

$$p_2 = \{D, E, F\}$$

$$p_3 = \{G, H, I\}$$

$$p_4 = \{J, K, L\}$$

In this example, the number of different union sets, s_{2j}, that can be made by combining 3 of the 4 partial subsets is m = 4. In general, the value for m can be determined from the equation m = (k choose v) = k!/(v! x (k-v)!), where k is the number of non-overlapping partial subsets and v is the number of reactive centers on the core molecule. Here, m = (4 choose 3) = 4!(3! x 1!) = 4. The 4 secondary subsets, s_{2j}, are:

$$s_{2,1} = p_1 \cup p_2 \cup p_3 = \{A, B, C, D, E, F, G, H, I\}$$

$$s_{2,2} = p_1 \cup p_2 \cup p_4 = \{A, B, C, D, E, F, J, K, L\}$$

$s_{2,3} = p_1 \cup p_3 \cup p_4 = \{A, B, C, G, H, I, J, K, L\}$

$s_{2,4} = p_2 \cup p_3 \cup p_4 = \{D, E, F, G, H, I, J, K, L\}$

In this example, the original set of 12 adduct types has been reduced to four subsets of 9 adduct types. Most importantly, all combinations of three adducts that can be chosen from the original primary set, s_1, are present in at least one of the secondary subsets, $s_{2,j}$. Accordingly, four adduct reagent mixtures would be prepared and separately reacted with the core molecule to produce four different product mixtures for screening. Each secondary product mixture would contain $9^3 = 729$ products, compared to the primary product mixture (using all twelve adducts) which would contain $12^3 = 1728$ products. After screening the four mixtures and identifying the most active mixture, a series of 4 smaller tertiary adduct subsets could be defined to continue the process.

To identify an active compound in a product mixture using fewer iterations, a higher value of k is favored. The above example can be repeated for k = 6 and v = 3.

$s_1 = \{A, B, C, D, E, F, G, H, I, J, K, L\}$

$p_1 = \{A, B\}$

$p_2 = \{C, D\}$

$p_3 = \{E, F\}$

$p_4 = \{G, H\}$

$p_5 = \{I, J\}$

$p_6 = \{K, L\}$

The number of different union sets, $s_{2,j}$, that can be made by combining 3 of the 6 partial subsets is m = (6 choose 3) = 20. The 20 secondary subsets, $s_{2,j}$, are:

$s_{2,1} = p_1 \cup p_2 \cup p_3 = \{A, B, C, D, E, F\}$

$s_{2,2} = p_1 \cup p_2 \cup p_4 = \{A, B, C, D, G, H\}$

$s_{2,3} = p_1 \cup p_2 \cup p_5 = \{A, B, C, D, I, J\}$

$s_{2,4} = p_1 \cup p_2 \cup p_6 = \{A, B, C, D, K, L\}$

$s_{2,5} = p_1 \cup p_3 \cup p_4 = \{A, B, E, F, G, H\}$

$s_{2,6} = p_1 \cup p_3 \cup p_5 = \{A, B, E, F, I, J\}$

$s_{2,7} = p_1 \cup p_3 \cup p_6 = \{A, B, E, F, K, L\}$

$S_{2,8} = p_1 \cup p_4 \cup p_5 = \{A, B, G, H, I, J\}$
$S_{2,9} = p_1 \cup p_4 \cup p_6 = \{A, B, G, H, K, L\}$
$S_{2,10} = p_1 \cup p_5 \cup p_6 = \{A, B, I, J, K, L\}$
$S_{2,11} = p_2 \cup p_3 \cup p_4 = \{C, D, E, F, G, H\}$
$S_{2,12} = p_2 \cup p_3 \cup p_5 = \{C, D, E, F, I, J\}$
$S_{2,13} = p_2 \cup p_3 \cup p_6 = \{C, D, E, F, K, L\}$
$S_{2,14} = p_2 \cup p_4 \cup p_5 = \{C, D, G, H, I, J\}$
$S_{2,15} = p_2 \cup p_4 \cup p_6 = \{C, D, G, H, K, L\}$
$S_{2,16} = p_2 \cup p_5 \cup p_6 = \{C, D, I, J, K, L\}$
$S_{2,17} = p_3 \cup p_4 \cup p_5 = \{E, F, G, H, I, J\}$
$S_{2,18} = p_3 \cup p_4 \cup p_6 = \{E, F, G, H, K, L\}$
$S_{2,19} = p_3 \cup p_5 \cup p_6 = \{E, F, I, J, K, L\}$
$S_{2,20} = p_4 \cup p_5 \cup p_6 = \{G, H, I, J, K, L\}$

Here, the original set of 12 adduct types has been reduced to 20 subsets of 6 adduct types. From these secondary subset definitions, 20 adduct reagent mixtures would be prepared and separately reacted with the core molecule to produce 20 different product mixtures for screening. Each secondary product mixture would contain $6^3 = 216$ products, compared to the primary product mixture (using all twelve adducts) which would contain $12^3 = 1728$ products. After screening the 20 mixtures and identifying the most active mixture, a series of 20 smaller tertiary adduct subsets could be defined to continue the process.

In this example, where v=3 and k=6, one iteration of the screening process decreases the number of adducts per subset by the factor 1/2, and the number of products per mixture by 1/8. These factors would narrow an adduct list in significantly fewer steps. However, a larger number of product mixtures must be screened to achieve this

narrowing of the adduct list in fewer iterations. When the screening assays are automated and a 96-well microliter plate is employed, it is efficient to produce as many as 96 different product mixtures or more at each step.

The Table below provides some preferred values for the number of partial subsets, the number of secondary product mixtures and the reduction in the number of adduct types for each iteration when core molecules with two, three, four and five reactive functionalities are used.

Table

Reactive functionalities (v)	Partial Subsets (k)	Product Mixtures (m)	Reduction Factor (v/k)
2	4	6	1/2
2	6	15	1/3
2	8	28	1/4
2	10	45	1/5
3	6	20	1/2
3	9	84	1/3
3	12	220	1/4
4	8	70	1/2
5	10	252	1/2

The reduction factor (v/k) is the amount by which the number of adduct types are reduced after each step of screening. Preferred reduction factors are those having

reciprocals which are integers. Such reduction factors can be repeatedly and evenly applied to initial adduct sets. Particularly preferred combinations of v and k are the combination v=3, k=9; v=3, k=6; and the combination v=4, k=8. For these particularly preferred combinations, the number of product mixtures can be easily contained in a 96-well microtiter plate.

The vessels employed may be any convenient vessels, which provide for the desired volume. The core molecule will normally be present in an amount of from about 0.1 millimole to 10 millimole. Normally, each adduct molecule will be present in at least about 0.5 millimole, usually at least about 2 millimoles and can be up to 100 millimoles or more in each mixture, usually not exceeding about 50 millimoles, more usually not exceeding about 10 millimole in each mixture. As already indicated, the amount of individual compounds which will be produced will be related to the minimal activity of interest, sensitivity of the assay, ease and economics of synthesis, and the like. Reaction volumes will usually be at least about 1 ml, more usually at least about 5 ml, and may be 0.5 L or more, usually not exceeding about 100 ml. The concentration of the core molecule will generally be in the range of about 1 mM to 10M, more usually about 10 mM to 1 M. Generally, the core molecule will be in a mol ratio of about 0.02 to 1 to each of the adduct molecules, depending on the relative rate of each of the adduct molecules, the desired rate of reaction, the volume of the

reaction mixture, and the like. Solvents will be chosen in accordance with the nature of the reaction, where the solvents may be polar or non-polar organic or inorganic, combinations thereof, single or mixtures of solvents, and the like. Illustrative solvents include dimethylformamide, DMSO, ethyl ether, propanol, acetonitrile, toluene, anisole, acetone, and the like. The core molecule and adducts will be combined, either neat or in the presence of a solvent, to carry out the reaction to form the mixture. Temperatures will depend upon the particular reaction, generally ranging from about -10 to 100°C. The core molecule, when present initially, will generally be at a concentration in the range of about 1 mM to 1 M, more usually in the range of about 10 mM to 100 mM. The concentrations of the reactants may vary depending upon the manner and order of addition.

As indicated previously, it will usually be desirable to normalize the reactivity of the individual adducts with the functionalities of the core molecule. By normalization it is intended that the difference in rates between any two adducts be not greater than twelve-fold, preferably not greater than ten-fold, and more preferably not greater than about five-fold. Therefore, the concentrations will be selected, so that there will be a reasonable opportunity to prepare all possible per-substituted compounds, where the difference in rate will not result in substantial exhaustion of one or more adducts during a period where there has been little or no

reaction of one or more other adducts. The relative rates of reaction of the individual adducts can be readily determined by setting up a pairwise experiment in which two adducts are reacted with the core molecule. The relative rates of reaction of the adduct of interest is directly related to the factor which affords equimolar yields of the two aforementioned adducts. In this way, one can readily define the various ratios to be used in each of the vessels for production of a particular composition mixture. Desirably the yield range should not be greater than about 2 to 10, preferably not greater than about 2 to 5.

Another method for normalization is to choose one adduct reagent to be a "normalization standard," and for each of the other adduct reagents, prepare an equimolar binary mixture of that reagent and the normalization standard. Each binary adduct mixture is then reacted with the core molecule and the "relative reactivity" of each adduct relative to the normalization standard is readily determined from the product yields.

Various orders of addition may be employed, where the core molecule is added to the preformed mixture of adducts, the core molecule and adducts are added substantially simultaneously or the adducts are added to the core molecule at the same or different rates and at the same or different concentrations, to substantially maintain the normalized rate of reaction of the various adducts.

The adduct molecules will usually be employed in greater than stoichiometric amounts, (the number of moles being equivalent to the proportion of reactive functionalities which the adduct will react with based on the number of adduct molecules and the number of different adduct reagents), generally in at least 2 fold molar excess, usually at least about 5 fold molar excess, and may be about 75 fold molar excess or more, generally less than about 50 fold molar excess. By having adduct molecules with similar rates of reaction with the reactive functionalities, the amounts of each of the adduct molecules will be relatively similar.

The core molecule may be free in solution or be bound to a solid support, where the solid support may be resins, *e.g.* Merrifield or modified versions thereof, silica-based support, *e.g.* aminopropyl silica (ref. J. Am. Chem. Soc. 116:1135 [1994]), solid surfaces such as magnetic particles, porous glass beads, polyethylene pins, *etc.* One of the advantages of the subject methodology is to have all of the reactants in solution, so that homogeneous reactions in solution will be preferred. Where particles are employed, one may join the core molecule to the particle by a functionality which is readily cleavable, while retaining the various adducts. A wide variety of molecules can be cleaved preferentially, e.g., thiophenyl ethers or silyl compounds which may be cleaved by mercuric trifluoroacetate or fluorides (tetrabutylammonium

fluoride), nitrobenzyl ethers, which can be cleaved by photolysis, *etc.*

For the most part, the reaction will be carried out with agitation, so as to ensure a substantially uniform mixture. Agitation can be achieved by rocking, stirring, shaking, or other convenient means.

A wide variety of addition or condensation reactions may be employed to produce the products. Because of the versatility of the system, the adducts may have the same or different functionality, so long as the required normalization can be achieved. The functionalities on the adduct molecules may include such reactive groups as halogen, usually other than fluorine, where the halogen may be used in a Grignard (RMgX) or Reformatsky (RZnX) reaction, oxy, oxo, ketone or aldehyde, carboxylic acids and derivatives thereof such as acid halides, esters and anhydrides, amino, hydrazino, ylides, cyanate, isocyanate, isothiocyanate, olefinic or acetylenic unsaturation, small alicyclic or heterocyclic rings, such as cyclopropyl, oxiranyl, and aziridinyl, phosphoric acids, such as phosphates, phosphinates, phosphinamides, phosphoryl halides, etc., sulfuric acid derivatives, including sulfonates, sulfates, and sulfinates, thiol, disulfide, nitriles, carbamates, thiocarbamates, imidate esters, and the like. Functionalities which may be produced include esters and amides, from both organic and inorganic acids, ethers, both oxy and thio, imines, hydrazones, chiral epoxides from the asymmetric oxidation of alkenes with an

enantioselective catalyst (J. Am. Chem. Soc. 116:6937-8 [1994]), etc.

The adduct molecules will be selected to substantially reduce or eliminate unimolecular or bimolecular side reactions, such as elimination, condensation, addition, and the like. For the most part, with active halides, elimination will be the major side reaction under basic or acidic conditions. Therefore, substitution at the α- or β-position will usually be limited to the presence of at least one hydrogen atom for aliphatic molecules and heterofunctionalities or other functionalities, which might aid elimination will be avoided at these positions. Ester and carbonyl groups will usually be avoided in basic media. The reactions of the common functionalities are well known and obvious side reactions can be avoided.

The core molecule may be aliphatic, alicyclic, aromatic or heterocyclic, where the heteroatoms will usually be nitrogen, oxygen and sulfur, although other heteroatoms, such as phosphorous, metal atoms, boron, etc. may be present. In some instances, metallocenes may serve as the core. Usually, there will be at least two active functionalities, more usually at least three active functionalities, and not more than about ten active functionalities, more usually not more than about six active functionalities, desirably 3 to 5, particularly 3 to 4. The active functionalities may be symmetrically situated, so as to have the same reactivity or may be

asymmetrically situated, so as to have differing activity. Compounds of interest include sugars, such as mono- and disaccharides, polyfunctionalized aromatic compounds, where the aromatic core may be carbocyclic or heterocyclic, such as benzene, naphthalene, biphenyl, pyridine, etc., may be alicyclic where the rings may be mono- or bicyclic or higher order, such as cyclohexane, cyclooctane, adamantane, bicyclo-heptane; acyclic organic compounds, such as ethylenediamine tetracetic acid, nitrolotriacetic acid, tris-hydroxyethylamine, 2,2,2-tri-thiolmethyl-1-methoxyethane, etc. To minimize steric interactions during the reaction and in the product, active functionalities on the core molecule will preferably be separated by a sufficient distance to minimize steric hindrance or other interactions which would substantially change the reactivity of an active functionality with the adduct reagents, either before the reaction of one of the active functionalities or after reaction of one of the reactive functionalities with an adduct reagent. While not universally true, two active functionalities will be separated by at least about 3 atoms and be bonded to an atom, usually carbon, which when saturated will usually be bonded to at least one hydrogen atom. Steric effects may be diagnosed by running reactions with a core molecule and a single adduct type and verifying that the main product is per-substituted.

While the core molecules may be symmetrical or asymmetrical, the symmetrical core molecules yield fewer

products, but the products are easier to separate and identify, as compared to asymmetrical molecules. For example, comparing 1,2,6-hexane and 1,3,5-trihydroxybenzene, the latter compound has both a C_3 and C_2 axis of symmetry. The addition of five adduct molecules to the asymmetrical 1,2,6-trihydroxyhexane should in principle lead to 5^3=125 distinct, trisubstituted products. The trigonal symmetry in 1,3,5-trihydroxybenzene reduces this to 35 as the number of distinct trisubstituted products is given by the formula, $(n^3 + 3n^2 + 2n)/6$.

In some situations, adduct molecules may be difunctional, where the adduct molecule can react with two functionalities on the core molecule to form a ring. In these situations, the core molecule will usually have two vicinal functionalities or be situated in spatial proximity, e.g. 1,8-disubstituted naphthalene, where the resulting rings will have from about 5 to 7 annular members. Illustrative situations include dihalides with glycols, activated dicarboxylic acids with diamines, diisocyanates with diamines, etc.

The linking group formed by the reaction between the core molecule functionality and the adduct functionality may take many forms, including carbon-carbon, carbon-oxygen, carbon-nitrogen and carbon-sulfur bonds. The reactions may be addition, substitution, elimination, condensation, free radical, or other reaction, as appropriate. Reactions which may be involved include

esterification, amidification, etherification, addition to unsaturation, Claisen condensation, metal catalyzed coupling reactions, asymmetric epoxidation or hydroxylation, *etc.* For carbon-carbon bond formation, one may use the Grignard reagent with an oxo or non-oxo-carbonyl functionality or the oxo functionality or an active halide with an organolithium compound. Suitable reactions may be found in March, "*Advanced Organic Chemistry*", 4th ed., Wiley-Interscience, NY, 1992, incorporated herein by reference. In particular, pages 417-424 relate to amide formation, page 903 to urea formation, pages 896-898 to imine formation, pages 386-387 to ether formation and pages 920-930 to Grignard reactions.

Reaction conditions will be optimized to provide for high yields of completely reacted core molecules, where all of the reactive functionalities have reacted with the adduct molecules. Conditions can be chosen to drive the reaction to completion, such as large excesses of the adduct molecules, elevated temperatures and pressures, long reaction times, where feasible, segregation of product from the main reaction mixture, catalysts, or the like. Segregation can be achieved where the final product has a different solubility from the core molecule and intermediate products, so that the final product may be taken up in a selective solvent. Functionalization of certain functionalities to enhance reactivity may be employed. With carboxylic acids, active esters may be

formed, e.g. pentafluorophenyl or o-nitrophenyl esters, or carbodiimides may be added. Lithium derivatives may be prepared from amides or amines. Metal ions may be added to enhance substitution reactions.

The creation of stereogenic centers via asymmetric induction with chiral catalysts may also be used. Thus, chiral secondary alcohols may be prepared from the enantioselective reduction of ketones, while highly enantioselective formation of epoxides can result from the oxidation of simple olefins in the presence of a chiral manganese catalyst.

At the end of the reaction, the reaction mixture may be subjected to a variety of treatments. Any particular treatment will depend upon the nature of the products, the purpose for which the product is to be screened, the solvents used, and the like. Any remaining adduct molecules may be removed by any convenient means. In some situations where an organic solvent is used, the solvent may be removed and the resulting product dispersed in an aqueous medium, an aqueous organic medium, or organic medium e.g., DMSO, DMF, ethanol, etc. Where the product has been produced bound to a surface, the product may be released from the surface as described previously, based upon the labile linking group. In some instances, small aliquots may be taken and analyzed in a gas chromatograph, GC-MS, capillary electrophoresis, or the like, so as to obtain some indication of the number of different compounds which have been produced. In some situations,

protective groups may have been employed with functionalities present on the adducts. The protective groups may be removed in accordance with conventional ways, depending upon the nature of the protective group, as well as the nature of the products. For example, amino groups may be protected with FMOC, where the FMOC groups may be removed with piperidine in DMF.

Various techniques may be employed to separate the products from the excess adduct molecules. Such techniques as distillation, chromatography, e.g. ion exchange chromatography, solvent extraction, base or acid extraction, or the like, may be employed in accordance with the nature of the products and adducts. If desired, the product mixture may be fractionated in accordance with a particular characteristic, to give more information about the nature of an active product.

After completion of the post processing of the product mixture, the product mixture is then screened for activity. The product mixture may be screened for one or more activities, depending upon the nature of the product.

One screening may be for binding affinity. Thus, the subject compositions may be screened for their ability to bind specifically with reasonable specificity to a target molecule. Thus, the subject compositions may be screened as enzyme inhibitors, as molecules for directing another molecule to a specific site, e.g. a physiological site, such as a blood protein, a surface membrane protein, specific organs, plant parts, pathogens, and the like. In

some instances, the subject compounds will be evaluated for activity, as cytotoxic agents, for inducing a signal across a surface membrane protein, as a competitor to a natural ligand, or the like. The subject compounds may also find use in diagnostics, where they may serve as reagents for competition with an analyte, binding to a ligand of interest, as labels, enzyme substrates, and the like. The subject compounds may also be evaluated for binding to nucleic acids, sugars, etc.

As illustrative of screening, for determining binding affinity, a number of different techniques may be employed. One may pass the mixture through a column in which the target molecule is bound to a solid support. From such a column, weakly binding or non-binding compounds will be eluted. After washing the column, one may then use an isocratic or gradient solvent with increasing polarity or increasing ionic strength, so as to obtain different fractions of compounds which bind, depending upon the effect of the solvent on the binding affinity. Where a fraction has one or few compounds, usually fewer than five, one may be able to analyze the compounds in the fraction to determine their structure. Even if a complete structure cannot be determined, information may be obtained so as to reduce the number of adducts required at the next stage.

One may do a gross determination by combining the product mixture with a labeled compound known to bind to the target. One can then determine whether there is one

or more compounds in the product mixture which can effectively compete with the labeled compound for the target. Thus, by employing a labeled compound, one can detect the amount of labeled compound which binds or does not bind in the presence and the absence of the product mixture. Where an enzyme is the target, one can combine the enzyme with the product mixture and its substrate and determine the enzymatic rate. A reduction in rate will indicate that one or more components of the product mixture are capable of inhibiting, competitively or non-competitively, the enzyme. Other techniques may also be employed.

For biological activity, one may screen the subject compounds for cytotoxic or stimulating effect. By combining the product mixture with a target unicellular microorganism in an appropriate medium, one can determine the rate of proliferation of the microorganism in the presence or absence of the product mixture. Stimulation or inhibition may be determined. Other screens may include binding to mammalian cells, where the target may be associated with homing, transduction of the signal across the membrane, inhibition of T-cells, binding to MHC antigens, etc.

In addition, the subject compounds may be screened for a wide variety of applications as additives, for fuels, oils, hydraulic fluids, boilers, plastics, food, cosmetics, etc., as pesticides, stabilizers, antioxidants, etc.

In each case, the mixture will be employed in accordance with conventional methods for evaluating a particular performance.

Where a product mixture has been found to be active, the process is then repeated, where a plurality of vessels are employed, but the primary set of adduct molecules is now up to and including the total number used for a particular vessel in the previous stage, where the product mixture from the vessel is found to be active. Thus, the number of vessels required and the adducts introduced in each vessel may be substantially reduced or expanded, as compared to the first stage, depending upon how few compounds are to be produced in each vessel. This iterative process may be repeated as many times as necessary, until one obtains a single compound or a mixture of compounds which can be individually analyzed.

The following examples are offered by way of illustration and not by way of limitation.

EXPERIMENTAL

EXAMPLE 1

Reaction of Resorcinol with Alkyl Halides

General

Unless otherwise indicated all reagents were obtained commercially and used as received without further purification. Neutral alumina, purchased from Aldrich, was of Brockmann I activity, ~150 mesh, and used as

obtained. Anhydrous potassium fluoride (KF, Baker grade) was handled under nitrogen, in an aqueous acid-free environment. The GC-MS system used for analysis consisted of (a) a Hewlett-Packard 5890 Series II Plus GC equipped with an HP-5 cross-linked 5% phenyl methyl silicone capillary column (0.25 mm i.d. x 30.0 mm long), interfaced to (b) a Hewlett-Packard 5972A Mass Selective Detector (MSD) equipped with a quadrupole mass filter. The data was processed with the aid of the MS (DOS) Chemstation software. The internal standard used in the gc analysis was an aromatic ether, p-tolyl ether (m/e 198amu).

Preparation of KF/Al_2O_3

An aqueous solution (200 mL) of KF (1.15 mol, 67g) was slowly poured into a 500 mL round-bottom flask containing neutral Al_2O_3 (100g). After about 15 min. of agitation to ensure thorough mixing, the resulting mixture was subjected to rotary evaporation at 50-60°C until most of the water had been removed. The now impregnated alumina was next heated in an oil bath kept at ~85 °C under higher vacuum (10^{-3} Torr) for an overnight (~12h) period. The KF/Al_2O_3 thus prepared can be readily handled in the open atmosphere and is indefinitely stable when stored in a desiccator.

Combinatorial Synthesis of a Sample Library of Ether Derivatives of Resorcinol

In a typical preparation, a 50 mL round bottom flask equipped with a magnetic stir bar and addition funnel was charged with resorcinol (1 mmol, 0.11g) dissolved in DMF (25 mL). This was followed by addition of the alkylating agent, KF/Al_2O_3 (10 mmol, 5 mmol/OH equiv., 1.60g). A mixture of three primary alkyl bromides, 1-bromopropane (A, 15.4 mmol), 4-bromobutyl acetate (B, 10.0 mmol), and β-bromophenetole (C, 24.2 mmol), was subsequently added dropwise from the addition funnel to the rapidly stirring dark green-colored suspension. The progress of the reaction mixture was monitored and analyzed by GC-MS until a satisfactory distribution of equimolar yields for the desired products was obtained (48h). The identity of the eluted peaks was established by electron-impact mass spectrometry (EI-MS) showed the molecular ion peak for all compounds of interest, with significantly smaller amounts of mono-O-alkylated derivatives of resorcinol, with no evidence of side reactions leading to unwanted products. Integration of the parent peaks of interest affords the relative yields of all six bis-O-alkylated products (Table 1). At this time, the reaction mixture was evaporated to dryness to remove excess $R'CH_2Br$ and other volatiles. The residue was extracted with 1:1(v/v) $Et_2O:CH_2Cl_2$ (4 x 20 mL) followed by filtration. The filtrate was evaporated to dryness to afford the desired ether derivatives of resorcinol as powders.

TABLE 1

Compound	Ion yield area/10^6	Ion, % total	Total ion yield	M.W. (amu)	Rel. molar yield
AA	0.970	0.094	10.32	194	0.053
AB	3.558	0.094	37.85	266	0.142
AC	1.464	0.089	16.45	272	0.060
BB	3.206	0.094	34.11	338	0.101
BC	2.527	0.086	29.38	344	0.085
CC	0.250	0.065	3.85	350	0.011

$R = [AB]/[CC] = 13$

The presence of a two-fold axis of symmetry in resorcinal implies that **AB** = **BA**, etc. For these products, the relative yields should be twice that for **AA**, **BB**, etc., so that the yield ratio (R) of the most abundant product to the least abundant product should be 2, as compared to the observed ratio of 13. By assuming that the reaction takes place in two alkylation steps, with the rate of each step proportional to: (i) the relative concentration of the alkyl bromide $[B]_{rel}$; and (ii) the relative "reactivity" of the alkyl bromide (k_B). Fitting mathematical expressions corresponding to the yield data in Table 1 are then written as shown in Table 2.

Table 2

Compound	Rel. molor yield, normalized to AA	Fitting expression	Best fit yields
AA	1.00	$[A]^2k_A^2$	1.00
AB	2.68	$2[A][B]k_Ak_B$	2.80

AC	1.13	$2[A][C]k_Ak_C$	1.16
BB	1.91	$[B]^2k_B^2$	1.96
BC	1.60	$2[B][C]k_Bk_C$	1.62
CC	0.21	$[C]^2k_C^2$	0.33

By simultaneously using these fitting expressions for each of the products and solving for the relative constants k_B and k_C (k_A being set to 1), the values of 1.539 and 0.636 are obtained, respectively. When these values of k_B and k_C are used in all six fitting expressions, the resulting best fit yields agree remarkably well with the measured relative yields. According to the method described above, using [A]:[B]:[C] = 15.4 mmol: 10.0 mmol: 24.2 mmol would be expected to lead to an equimolar distribution product yield, with R = 2. In a reaction mixture with the alkyl bromides having the concentrations indicated above, the relative yields of the six products of interest indicated an essentially equimolar distribution of product yields (R = 2.56). This is excellent agreement with theoretical, where the influence of the first O-alkylation on the second O-alkylation is being ignored.

EXAMPLE 2

Combinatorial Synthesis of a Sample Library of Ether Derivatives of Phloroglucinol

In a typical preparation, a 100 mL round bottom flask was charged with phloroglucinol dihydrate (1 mmol, 0.16g) dissolved in DMF (25 mL) followed by addition of KF/Al_2O_3 (15 mmol, 2.4g). To the rapidly stirring gray-colored suspension, a mixture of three alkyl bromides: in a first study A (23 mmol), B (15 mmol) and C (36 mmol); and in a second study, A (23 mmol, 2.8g), B (18 mmol, 3.5g) and C (73 mmol, 14.7g) (wherein A, B, and C are as defined in Example 1), was added dropwise via an addition funnel. After 48h, GC-MS analysis of an aliquot sampled from the yellow-green reaction mixture showed a satisfactory distribution of yields for the desired tri-O-alkylated products. The mixture was evaporated to dryness and worked up as described above.

In the first study CCC could not be detected, which may have been a result of decomposition during the gc detection. Upon readjustment of the relative concentrations to enhance the production of CCC, the presence of CCC could now be detected. Following the procedure described for modifying the concentrations with resorcinol as the core compound, the cube root of [AAA]/[BBB] was taken to obtain the factor by which the current concentration of B needs to be multiplied such that [AAA] = [BBB], e.g. 1.2 x 15 mmol = 18 mmol; (ii) by obtaining the value of [BCB]/[BCC], which yields the factor by which the current concentration of C must be multiplied such that [BCB] = [BCC], and [BBB] =[AAA], e.g. 1.7 x 1.2 x 36 mmol = 73 mmol. The yields of the resulting

ten tri-O-alkylated products showed an improved R = 11, as compared to the previous R = 12.2. The results are reported for the first and second studies in Tables 4 and 5, respectively.

Table 3

Product	M+ yield (Area x 10^{-6})	M+% of total	Total ion yield	M.W. (amu)	Rel. molor yield	Rel. mol. yield, normalized to AAA
AAA	0.114	0.050	2.278	252	0.009	1.00
BAA	0.490	0.051	9.524	324	0.029	3.22
CAA	0.357	0.041	8.686	330	0.026	2.89
ABB	0.673	0.049	13.87	396	0.035	3.89
ABC	0.904	0.037	24.40	402	0.061	6.78
ACC	0.207	0.019	10.68	408	0.026	2.89
BBB	0.061	0.026	2.302	468	0.005	0.56
BBC	0.113	0.014	8.316	474	0.017	1.89
BCC	0.041	0.008	5.102	480	0.011	1.22

[ABC]/[BBB] = 12.2

Product	Mathematical fitting expression
AAA	$[A]^3k_A^3$
BAA	$3[A]^2[B]k_A^2k_B$
CAA	$3[A]^2[C]k_A^2k_C$
ABB	$3[A][B]^2k_Ak_B^2$
ABC	$6[A][B][C]k_Ak_Bk_C$

	ACC	$3[A][C]^2k_Ak_C^2$
	BBB	$[B]^3k_B^3$
	BBC	$3[B]^2[C]k_B^2k_C$
	BCC	$3[B][C]^2k_Bk_C^2$
5	CCC	$[C]^3k_C^3$

Table 4

Product	M+ yield (Area x 10^{-6})	M+% of total	Total ion yield	M.W. (amu)	Rel. molar yield	Rel. mol. yield, normalized to AAA
AAA	0.075	0.049	1.53	252	0.006	1.00
BAA	0.335	0.046	7.28	324	0.023	3.83
CAA	0.373	0.042	8.88	330	0.027	4.50
ABB	0.395	0.047	8.40	396	0.021	3.50
ABC	0.986	0.039	25.3	402	0.063	10.5
ACC	0.453	0.025	18.1	408	0.044	7.33
BBB	0.041	0.017	2.43	468	0.005	0.90
BBC	0.168	0.015	11.2	474	0.024	4.00
BCC	0.170	0.012	14.2	480	0.030	5.00
CCC	0.022	0.005	4.40	486	0.009	1.52

EXAMPLE 3

Mixed O-Alkylations of Resorcinol

The adduct reagents were primary alkyl bromides: bromopropane (A); 3-acetoxypropyl-1 bromide (B); phenoxyethyl bromide (C); D-citronellyl bromide (D); and 3-cyanopropyl-1 bromide (E). The reaction was carried out as described in Exaple 1. Mixed derivitizations were performed, by combining resorcinol pairing each of the alkyl bromides with A to determine the relative reactivity under the reaction conditions for each of the alkyl bromides normalized to the reactivity of A. The weight ratios of the reactants in the reaction mixture were:

Alkyl Bromide	Relative Reactivity	Amount used mmol
A	1	10
B	1.5	6.7
C	0.8	12.5
D	0.56	17.7
E	3.7	2.7

Using the rate-adjusted quantities of the alkyl bromides with 1 mmol of resorcinol, the reaction was carried out for 24h at a temperature of 20°C to produce the expected 15 products. The following table indicates the results. The presence of the products and their amounts were determined by a total ion chromatography and mass spectroscopy. Two of the products, BD and CE, had identical retention times on the total ion chromatogram and formed a single large peak at 13.73 min. Single ion monitoring was used to determine the individual yields of BD and CE. As seen in the table, the relative yield of the 15 products fell within a 3.63 range. This is not far

from the ideal range of 2. No significant amount of products from side reactions were observed in the analyses of the reaction mixture.

Product (MW,amu)	Retention time (min.)	M^+ yield (Area/10^6)	M^+, % of total	Total M^+ yield	Relative molar yield	Rel. mol. yield, normalized to AA
AA (194)	8.30	0.284	0.111	2.56	0.013	1.00
BB (338)	13.23	0.321	0.054	5.91	0.017	1.34
CC (350)	14.99	0.323	0.072	4.48	0.013	1.00
DD (386)	14.18	0.182	0.022	8.10	0.021	1.61
EE (244)	12.38	0.579	0.104	5.55	0.023	1.75
AB (266)	11.05	0.512	0.071	7.21	0.027	2.09
AC (272)	12.06	0.554	0.087	6.41	0.024	1.81
AD (290)	11.66	0.329	0.035	9.48	0.033	2.51
AE (219)	10.43	0.621	0.087	7.14	0.033	2.51
BC (344)	14.12	0.879	0.082	10.72	0.031	2.38
BD (362)	13.73	0.475	0.037	12.88	0.036	2.74
BE (291)	12.80	0.751	0.061	12.32	0.042	3.26
CD (368)	14.58	0.464	0.039	11.76	0.032	2.46
CE (297)	13.73	0.943	0.073	12.88	0.043	3.34
DE (315)	13.32	0.304	0.021	14.85	0.047	3.63

Example 4

Determination of rate normalization constants for 10 R'Br with respect of phloroglucinol

The conditions for the normalization were 1 mmol phloroglucinol, 15 mmol of KF/Al_2O_3 15 mmol of propyl bromide and 15 mmol of the test bromide in DMF at 25°C. The following table indicates the results of the rate normalization of 10 aliphatic bromides.

	Test Bromide	Normalization factor
A	propyl	1
B	cyclopropylmethyl	2
C	oxiranylmethyl	3
D	2-methoxyethyl	2
E	3-cyanopropyl	0.23 (0.27*)
F	3-methylbutyl	1.67
G	hexyl	0.8
H	2-methoxyethoxy-ethylene	3
I	4-acetoxybutyl	0.67

J	citronellyl	1.60 (1.84*)

* with respect to resorcinol

II. Reaction of Phloroglucinol with Primary Set of 10 Rate Adjusted R'Br

v + 3, n = 10

Total number of compounds possible is 220.

R'Br	mL	mmol
A	0.43	4.71
B	0.91	9.42
C	1.21	14.13
D	0.89	9.42
E	0.12	1.10
F	0.94	7.85
G	0.53	0.43
H	1.92	14.13
I	0.454	3.14
J	1.49	7.54
Total	8.88	75.2

Using the GC-MS analysis described previously, molecular ions were detected for all of the possible products except for DDD, EEE, JJJ, and DEJ, where the peaks may have been obscured by a stronger peak from another compound. In other less complex preparations, these compounds were observed. The most abundant products were BGI (17.89 min), DHJ (19.13 mn), FGI (17.78 min), EFJ and FHJ (19.26 min), EFI and FHI (18.50 min), BEI and FGJ (18.60 min), ABJ and AEH (16.73 min).

The experimental ratio of BGI/GGG was 17, where the ideal equimolar yield predicts a ratio of 6. The deviation from the ideal was 2.8

It is evident from the above results, that one can produce fairly sophisticated libraries very quickly and easily, which allow for screening of the resulting mixtures. Where a mixture is found to be active, if the mixture is too complex to be analyzed directly, a second round of reactions is carried out, so that ultimately one can define the compound(s) present in the mixture which provides the activity. The method allows for using reactions in solution, so as to avoid the various problems associated with reactions on surfaces. In addition, it allows for great versatility and type of compounds which can be produced, so as to provide for high probabilities of defining useful compounds associated with a particular application.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

WHAT IS CLAIMED IS:

1. A method for preparing a large number of related compounds in a plurality of complex mixtures, which method allows for the screening of the mixtures as to the identity of compounds having a designated characteristic, said method comprising:

as a first or intermediate stage, introducing under reaction conditions into each of at least three vessels a common polyfunctionalized core molecule and a first set of adduct molecules which react with said core molecule, where each first set of adduct molecules is characterized by being different from the other first sets, overlapping, having fewer than the total number of adduct molecules and the combined products from each first set includes substantially all possible per-substituted core molecules, to provide at least three first product mixtures;

screening each of said first product mixtures for a designated activity to provide a first set active mixture;

as a next stage, introducing under reaction conditions into each of at least three vessels said common polyfunctionalized core molecule and a second set of adduct molecules which react with said core molecule, where each second set is characterized by consisting of members of said first set active mixture, being different from the other second sets, overlapping, having fewer than the total number of adduct molecules of said first set active mixture and the combined products from each second

set includes substantially all possible per-substituted core molecules, to provide at least three second product mixtures;

screening each of said second product mixtures for a designated activity to provide a second set active mixture; and

optionally repeating said next stage and screening until at least one compound is characterized as having said designated activity.

2. A method according to Claim 1, wherein the relative concentrations of each of the adduct molecules in each of the sets results in substantially equivalent concentrations of each of the products.

3. A method according to Claim 1, wherein said core molecule has from 2 to 5 of the same functional groups and the number of vessels is at least 5.

4. A method according to Claim 3, wherein the number of adduct molecules in each vessel is at least 6 in the first stage.

5. A method according to Claim 1, wherein said screening is for binding to a protein.

6. A method according to Claim 1, wherein each of said adduct molecules is in substantial excess to stoichiometry.

7. A method for preparing at least 100 related compounds in a plurality of complex mixtures, which method allows for the screening of the mixtures as to the identity of compounds having a designated characteristic, said method comprising:

as a first or intermediate stage, introducing under reaction conditions into each of at least three vessels a common polyfunctionalized core molecule having the same functional groups and a first set of adduct molecules which react with said core molecule, where each first set of adduct molecules is characterized by being derived from a union of subset units, different from the other first sets, overlapping, having fewer than the total number of adduct molecules, the relative concentrations of each of the adduct molecules in each of the sets results in substantially equivalent concentrations of each of the products, and the combined products from each first set includes substantially all possible per-substituted core molecules, to provide at least three first product mixtures, wherein each subset unit is non-overlapping with other subset units, the number of subsets is or greater than the number of functionalities on said core molecule, and wherein each set has an equal number of at least two subsets and fewer than the total number of subsets;

screening each of said first product mixtures for a designated activity to provide a first set active mixture;

as a next stage, introducing under reaction conditions into each of at least three vessels said common polyfunctionalized core molecule having the same funcional groups and a second set of adduct molecules which react with said core molecule, where each second set is characterized by consisting of members of said first set active mixture, being different from the other second sets, overlapping, having fewer than the total number of adduct molecules of said first set active mixture and the combined products from each second set includes substantially all possible per-substituted core molecules, to provide at least three second product mixtures;

screening each of said second product mixtures for a designated activity to provide a second set active mixture; and

optionally repeating said next stage and screening until at least one compound is characterized as having said designated activity.

8. A method according to Claim 7, wherein said core molecule is functionalized with oxy, oxo, amino thiol, nitrile, isocyanate, isothiocyanate, carbamate, thiocarbamate or hydrazine groups.

9. A method according to Claim 8, wherein said core molecule has an aromatic core and is symmetrical as to the functional groups.

10. A method according to Claim 7, wherein said core molecule has from 2 to 5 functional groups and the number of vessels is at least 6.

11. A method according to Claim 7, wherein the number of adduct molecules in each vessel is at least 6 in the first stage.

12. A method according to Claim 7, wherein said screening is for binding to a protein.

13. A method according to Claim 7, wherein each of said adduct molecules is in substantial excess to stoichiometry.

14. A method for preparing at least 1000 related compounds in a plurality of complex mixtures, which method allows for the screening of the mixtures as to the identity of compounds having a designated characteristic, said method comprising:

as a first or intermediate stage, introducing under reaction conditions into each of at least three vessels containing a common polyfunctionalized core molecule a first set of adduct molecules which react with said core

molecule, where each first set of adduct molecules is characterized by being derived from a union of subset units, different from the other first sets, overlapping, having fewer than the total number of at least 12 adduct molecules, the amounts of each of said adduct molecules being in substantial stoichiometic excess, the relative concentrations of each of the adduct molecules in each of the sets results in substantially equivalent concentrations of each of the products, and the combined products from each first set includes substantially all possible per-substituted core molecules, to provide at least four first product mixtures, wherein each subset unit is non-overlapping with other subset units, the number of subsets is greater than the number of functionalities on said core molecule, and wherein each set has an equal number of at least two subsets and fewer than the total number of subsets;

screening each of said first product mixtures for a designated activity to provide a first set active mixture;

as a next stage, introducing under reaction conditions into each of at least three vessels said common polyfunctionalized core molecule and a second set of adduct molecules which react with said core molecule, where each second set is characterized by consisting of members of said first set active mixture, being different from the other second sets, overlapping, having fewer than the total number of adduct molecules of said first set active mixture and the combined products from each second

set includes substantially all possible per-substituted core molecules, to provide at least three second product mixtures;

screening each of said second product mixtures for a designated activity to provide a second set active mixture; and

optionally repeating said next stage and screening until at least one compound is characterized as having said designated activity.

15. A method for preparing a large number of related compounds in a plurality of complex mixtures, which method allows for the screening of the mixtures as to the identity of compounds having a designated characteristic, said method comprising:

as a first or intermediate stage, introducing under reaction conditions into each of at least three vessels a common polyfunctionalized core molecule bonded by a cleavable linkage to a solid support and a first set of adduct molecules which react with said core molecule, where each first set of adduct molecules is characterized by being different from the other first sets, overlapping, having fewer than the total number of adduct molecules and the combined products from each first set includes substantially all possible per-substituted core molecules, to provide at least three first product mixtures;

screening each of said first product mixtures for a designated activity to provide a first set active mixture,

while said product mixture is bound to said solid support or released from said solid support;

as a next stage, introducing under reaction conditions into each of at least three vessels said common polyfunctionalized core molecule bound to a solid support and a second set of adduct molecules which react with said core molecule, where each second set is characterized by consisting of members of said first set active mixture, being different from the other second sets, overlapping, having fewer than the total number of adduct molecules of said first set active mixture and the combined products from each second set includes substantially all possible per-substituted core molecules, to provide at least three second product mixtures;

screening each of said second product mixtures for a designated activity to provide a second set active mixture, while said product mixture is bound to said solid support or released from said solid support; and

optionally repeating said next stage and screening until at least one compound is characterized as having said designated activity.

16. A method according to Claim 15, wherein said solid support is a particle.

17. A method according to Claim 15, wherein said solid support is a vessel wall.

18. A combinatorial library produced according to the method of introducing under reaction conditions into each of at least three vessels a common polyfunctionalized core molecule and a first set of adduct molecules which react with said core molecule, where each first set of adduct molecules is characterized by being different from the other first sets, overlapping, having fewer than the total number of adduct molecules and the combined products from each first set includes substantially all possible per-substituted core molecules, to provide at least three first product mixtures.

19. A combinatorial library produced according to the method of introducing under reaction conditions into each of at least three vessels a common polyfunctionalized core molecule having the same functional groups and a first set of adduct molecules which react with said core molecule, where each first set of adduct molecules is characterized by being derived from a union of subset units, different from the other first sets, overlapping, having fewer than the total number of adduct molecules, the relative concentrations of each of the adduct molecules in each of the sets results in substantially equivalent concentrations of each of the products, and the combined products from each first set include substantially all possible per-substituted core molecules, to provide at least three first product mixtures, wherein each subset unit is non-overlapping with other subset

units, the number of subsets is greater than the number of functionalities on said core molecule, and wherein each set has an equal number of at least two subsets and fewer than the total number of subsets.

20. A combinatorial library according to Claim 19, wherein said core molecule is functionalized with oxy, oxo, amino thiol, nitrile, isocyanate, isothiocyanate, carbamate, thiocarbamate or hydrazine groups.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/12956

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :G01N 33/52; C12Q 1/00
US CL :435/7.1; 436/501, 518, 35, 36

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/7.1; 436/501, 518, 35, 36

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN, APS
search terms: structure search, combinatorial, library, array

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO, A, 86/00991 (GEYSEN) 13 FEBRUARY 1986, PAGE 4, LINE 16-PAGE 6, LINE 4	1-20
Y	WO, A, 91/18598 (LIU ET AL.) 12 DECEMBER 1991, SEE PAGE 5.	1-20
Y,P	JOURNAL OF MEDICINAL CHEMISTRY, VOLUME 37, NUMBER 10, ISSUED 13 MAY 1994, GORDON ET AL., "APPLICATIONS OF COMBINATORIAL TECHNOLOGIES TO DRUG DISCOVERY. 2. COMBINATORIAL ORGANIC SYNTHESIS, LIBRARY SCREENING STRATEGIES, AND FUTURE DIRECTIONS", PAGES 1385-1401, SEE ENTIRE ARTICLE.	1-20

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
26 JANUARY 1995

Date of mailing of the international search report
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/12956

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ACCOUNTS OF CHEMICAL RESEARCH, VOLUME 11, ISSUED 1978, LEZNOFF, "THE USE OF INSOLUBLE POLYMER SUPPORTS IN GENERAL ORGANIC SYNTHESIS", PAGES 327-333, SEE PAGE 327.	12-15